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SYMPTOMATIC AND ETIOLOGIC RELATIONS OF THE CANKER AND THE BLOSSOM BLAST OF PYRUS AND THE BACTERIAL CANKER OF PRUNUS[1]

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INTRODUCTION

IN 1931 a heretofore undescribed bacterial canker of pear trees was found in Sierra Nevada foothill orchards. A brief discussion of symptoms, giving results of inoculations and comparing the disease with fire blight, *Erwinia amylovora* (Burrill) Bergey *et al.*, was published in 1934.[18] [3] The causal organism was not described except as it differed from *Erwinia amylovora* in producing a greenish pigment on many media, thus allying itself with *Phytomonas cerasi* (Griffin) Bergey *et al.*, cause of the stone-fruit bacterial canker.[17]

A blossom blast of pear in California differing from that caused by fire blight was briefly described by Thomas and Ark,[15] [4] who report the causal organism as similar to those of citrus blast and stone-fruit canker.

The orchards in which the writer first found the limb-canker disease have remained free of blossom blast, though planted with Beurre Bosc, a variety elsewhere susceptible to blossom infection. Limb and blossom symptoms in the trees growing in other districts indicate that all are phases of the same disease. One purpose of this work, therefore, was to compare the bacteria obtained from these parts of the host.

Reports from New York[3, 4] and Arkansas[9, 10] regarding infection of pear leaves, fruit, and blossoms by bacteria possessing cultural char-

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[3] Superscript numbers in parentheses refer to Literature Cited at end of this paper.

[4] Thomas and Ark designated the blossom disease "blast" to distinguish it from that caused by *Erwinia amylovora*. Rosen[10] in Arkansas had used the term earlier to designate a pear-blossom blight caused by an organism similar to *Phytomonas citriputeale* (C. O. Smith) Bergey *et al.*, the cause of citrus blast.

acteristics similar to these organisms were additional reasons for the study.

Evidence obtained earlier[18] pointed to similarities between the California pear-canker organism and *Phytomonas cerasi,* cause of the stone-fruit canker. This study, therefore, also includes symptomatic and etiologic comparisons of the two diseases.

COMPARISON OF THE PEAR AND STONE-FRUIT DISEASES

The bacterial canker of stone-fruit trees is described in detail in an earlier publication.[17] Pear blossom blasts and limb cankers are briefly described by Thomas and Ark[15] and by the present writer.[18] Certain features of the pear disease not previously discussed are included herein.

Twig Infection.—In certain years an infection of small branches and twigs causes a considerable loss (fig. 1, *A* and *B*). Sloughing away of the periderm and a spongy condition of the cortex and outer phloem are characteristic symptoms present in all bark cankers whether in large or in small branches. Although differing in external appearance, the twig cankers of the pear and stone-fruit diseases have a similar manner of involving the tissues of the bark. This point will be discussed later.

Dormant-Bud Blast.—Figure 1, *C* and *D,* shows small lesions surrounding dormant buds. Both blossom and leaf buds are susceptible to infection and are points from which the disease enters and kills small branches. *Phytomonas cerasi* causes a similar infection of the dormant buds of stone-fruit trees. Twigs as well as small branches are killed by the extension of these infections.

Infection of the Fruit-Cluster Base.—A phase of the pear disease that has no counterpart in the stone-fruit disease is the infection of the fruit-cluster bases. Although the exact time of infection is not known, presumably the bacteria enter the fruit-stem scars after the fruit is picked.

Limb Cankers.—Branch cankers, adequately pictured in the earlier article,[18] were the most common symptoms in the pear orchards where the disease was first found. New cankers and the active margins of old ones present the same appearance as the twig infection shown in figure 1, *A* and *B*. The centers of old cankers are characterized by a longitudinal and transverse cracking of the periderm and by a gradual sloughing away of affected cortex. In many cases where the disease does not at once extend to the cambium, the underlying healthy tissue, in forming a new periderm, forces the diseased tissue outward. Branches diseased for a few years will, therefore, become roughened before natural longitudinal suturing begins. Figure 2 shows a tree in which the bark of certain limbs is rough whereas that of others is smooth.

Branch cankers of the pear and stone-fruit diseases have very similar internal characteristics. In both cases the margins of the affected area are made up of numerous, loosely knit streaks, the paths along which

Fig. 1.—Bacterial canker on small branches of pear: *A* and *B*, young terminal shoots with characteristic sloughing away of periderm; *C* and *D*, infection of dormant buds and accompanying lesions in the twig.

bacterial invasion has progressed. On both hosts the streaks of active cankers are light brown and water-soaked. When inactive the streaks are dark brown to black on pear and brown to reddish brown on stone fruits.

Blossom Blast.—Thomas and Ark[15] found that the pear blossom blast closely resembled blight, caused by *Erwinia amylovora,* but is distinguishable because blast seldom extends more than 1 to 2 inches into the

Fig. 2.—Bacterial canker on trunk and scaffold branches of Wilder pear. The longitudinal and transverse cracking of periderm does not occur on all the branches.

spur and never involves a bacterial exudate. Figure 3 of the present article shows the withered, blackened blossoms and leaves of an infected spur. Arrows indicate the limits of the canker in the branch.

Cherry and apricot blossoms are sometimes blighted by *Phytomonas cerasi,*[17] the general symptoms being the same as those of pear blast.

Leaf and Fruit Infection.—The disease has not been observed on pear leaves in California, but apricot and cherry leaves are frequently attacked by *Phytomonas cerasi.*

No natural infection of pear fruit has been noted in California. Fruit infection by inoculation has, however, been obtained (fig. 6). In the only case of fruit infection by *Phytomonas cerasi* definitely established, small,

superficial, black pits appeared on the surface of green apricots. Infection has, however, been secured on apricot and plum fruit by needle-puncture inoculations with *P. cerasi*, the resulting symptoms being small, black, sunken pits like those from natural infection.

Fig. 3.—Blossom blast of pear. Arrows point to the margins of the diseased area in the twig. This is usually as far as blossom blast progresses the first year.

Other Points of Similarity Between the Pear and Stone-Fruit Diseases.—The pear canker disease differs from fire blight in being active during fall, winter, and early spring, when blight is inactive.[18] A similar seasonal nature of stone-fruit bacterial canker has been observed.

To summarize: the pear and stone-fruit diseases possess marked similarities in the parts of trees they attack, in appearance of the invaded tissue, and in their activity during the same seasons of the year.

PATHOGENICITY STUDIES

The primary object of the inoculation work was to compare bacteria from the pear canker and blossom blast with the organism of stone-fruit canker (*Phytomonas cerasi*). Incidentally, certain similar organisms attacking the pear in other sections of the country, together with the citrus-blast bacterium (*P. citriputeale*), were included in these tests. The history of the cultures, so far as available, is given in table 1.

TABLE 1

History of Cultures Used in Inoculation and Cultural Experiments

Organism or culture number	Isolation history		Source of culture		
	Isolated by	Date	State	Host	Diseased part of host
Phytomonas cerasi var. *prunicola* I	Author	Aug. 8, 1930	Calif.	Plum	Limb canker
Phytomonas cerasi var. *prunicola* II	Author	Mar. 21, 1932	Calif.	Apricot	Blossom blight
Phytomonas cerasi I	Author	Feb. 22, 1930	Calif.	Apricot	Limb canker
Phytomonas cerasi II	Author	Apr. 10, 1933	Calif.	Peach	Limb canker
Wilder I	Author	Jan. 1, 1931	Calif.	Pear	Limb canker
Wilder II	Author	Mar. 7, 1933	Calif.	Pear	Blight of blossom base
Wilder III	Author	Aug. 12, 1932	Calif.	Pear	Limb canker
Bartlett I	P. A. Ark	May 15, 1932	Calif.	Pear	Blossom blight
Winter Nelis I	P. A. Ark	Apr. 24, 1933	Calif.	Pear	Blossom blight
Winter Nelis II	P. A. Ark	Apr. 24, 1933	Calif.	Pear	Twig blight
Apple I	Author	Apr. 28, 1933	Calif.	Apple	Limb canker
Apple II	Author	Apr. 28, 1933	Calif.	Apple	Limb canker
Phytomonas utiformica "r"	F. Clara	July, 1931	N. Y.	Pear	Blossom blight
Phytomonas utiformica "f"	F. Clara	July, 1931	N. Y.	Pear	Blossom blight
Arkansas I	H. R. Rosen	.....	Ark.	Pear	Blossom blight
Arkansas II	H. R. Rosen	.....	Ark.	Pear	Blossom blight
Phytomonas citriputeale	Author	Mar. 14, 1932	Calif.	Orange	Twig blast
Phytomonas papulans	J. W. Roberts	.....	East. U. S.	Apple	Target canker

Eighteen different cultures were employed at various times. Two were cultures of *Phytomonas cerasi* var. *prunicola:* two were *P. cerasi:* and three were cultures the author had isolated from limb cankers of Wilder pear from an orchard in Placer County (Wilder I, II, III). Three (Bartlett I, Winter Nelis I and II) were furnished by P. A. Ark, who had obtained them from blossom and twig blight of pears from El Dorado, San Benito, and Santa Cruz counties, respectively. Two cultures from apple were obtained from Mendocino and Sonoma counties. The two of *Phytomonas utiformica* Clara (isolates "r" and "f") were sent by Clara[4] to Harvey E. Thomas of this Station. Unfortunately, isolate "r" was lost after the first two series of inoculations in 1933. H. R.

Rosen[10] furnished two cultures designated "receptacle" and "petal" (Arkansas I and II, respectively). The culture *P. citriputeale* was obtained from twig blast of orange in Placer County. Inoculations of this organism into lemon and orange fruits produced the sunken lesions typical of the black-pit disease in nature. John W. Roberts furnished the culture that he provisionally called *P. papulans,*[7] the organism originally described by Rose[8] as the cause of apple blister spot. Roberts obtained this culture from the target canker of apples.

Inoculations were made by first piercing the bark tangentially, then injecting into the holes a drop of the organism in water suspension.

TABLE 2

RESULTS OF INOCULATING WILDER PEAR TREES JANUARY 20, 1933

Organism	Inoculations producing symptoms, per cent	Length of cankers, mm*
Phytomonas cerasi var. *prunicola* I	77	15– 25†
Wilder I	92	25–102
Phytomonas utiformica "r"	88	25– 64
Phytomonas utiformica "f"	100	25–127
Controls	5‡	5– 10

* Measurements made 62 days after inoculation.

† Later observations showed that these cankers continued to extend until they involved considerably more area.

‡ Three infections of control wounds on one tree were clearly cases of secondary infections. The remaining 61 wounds were not infected.

From 50 to 75 inoculations were made from each culture used in each experiment into three to five different trees. Control punctures were made in different limbs of the same trees.

Although most of these organisms are similar in certain respects, some difference of opinion exists as to the closeness of the relation. The literature on this phase will be reviewed in a later section.

Results of Inoculations.—Table 2 shows the results of inoculations into pear during late winter of 1933. Cultures Wilder I and *Phytomonas utiformica* ("r" and "f") produced extensive cankers (fig. 4) in every way typical of those in nature. *P. cerasi* var. *prunicola* had produced small but definite cankers (fig. 5, *C* and *D*). As later observations showed, these cankers continued to extend and eventually became 6 or more inches long. A second series of inoculations made with Wilder I, *P. cerasi,* and *P. cerasi* var. *prunicola* into Wilder pear resulted in cankers from 3 to 6 inches long in all cases. No differences could be found between cankers produced by *P. cerasi* and Wilder I.

In January, 1933, Blenheim apricot limbs and Phillips Cling peach limbs, inoculated with the stone-fruit organisms, Wilder I culture, and

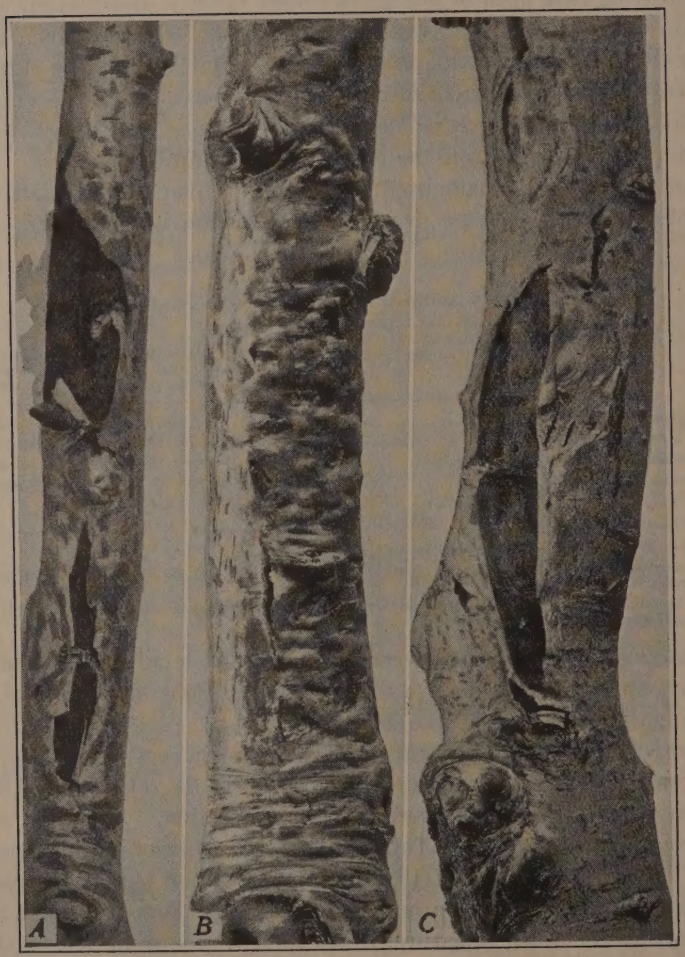

Fig. 4.—Symptoms produced by *Phytomonas utiformica* "r" (*A*), by *P. utiformica* "f" (*B*), and by Wilder I (*C*), on Wilder pear branches. Compare these symptoms with those from natural infections shown in figure 1.

Fig. 5.—Cankers produced by culture Wilder I on Wilder pear (*B*) and Blenheim apricot (*A*). On the apricot, the bark canker was 1½ inches long. *C* and *D* are cankers produced by *Phytomonas cerasi* var. *prunicola* on Wilder pear.

the two cultures of *Phytomonas utiformica,* developed definite cankers in every way typical of the bacterial canker of these hosts in nature (table 3). The fact that Wilder I produced somewhat smaller cankers than the stone-fruit organism may or may not be significant.

In February, 1934, when a series of inoculations were made into Duarte plum, conditions apparently did not favor rapid canker development, since cultures of *P. cerasi* and *P. cerasi* var. *prunicola* produced

TABLE 3

Results of Inoculating Apricot and Peach Trees January 7, 1933

Organism	Blenheim apricot		Phillips Cling peach	
	Inoculations producing symptoms, per cent	Length of cankers,* mm	Inoculations producing symptoms, per cent	Length of cankers,* mm
Phytomonas cerasi var. *prunicola* I	82	20–64	60	20–40
Phytomonas cerasi I	93	20–30	56	20–50
Wilder I	48	20–40	42	20–30
Phytomonas utiformica "r"	90	20–70	78	10–50
Phytomonas utiformica "f"	54	20–30	..	.....
Controls	0	0	0	0

* Measurements made 26 days after inoculation.

rather small lesions. Nevertheless, as table 4 shows, distinct symptoms were produced by the six cultures obtained from blossom blast, twig blight, and limb canker of pear in California. At least one culture from apple (Apple I), the culture of *Phytomonas utiformica,* and culture Arkansas I also produced distinct symptoms.

On January 21, 1935, inoculations were made into apricot, peach, sweet cherry, and plum. Eleven cultures were used, each being inoculated at 60 places on three trees of each species. As table 5 shows, on apricot and peach all cultures except *Phytomonas papulans* produced diseased areas as large as *P. cerasi* var. *prunicola* or larger. On cherry the diseased areas were smaller, but were otherwise indistinguishable from those produced by the stone-fruit organism. On plum, *P. citriputeale* and culture Winter Nelis I produced cankers somewhat larger than did *P. cerasi* var. *prunicola,* whereas the rest of the cultures, except *P. papulans,* produced cankers somewhat smaller. Thus. *P. papulans* was the only culture that was distinctive on these four hosts. Secondary infections, which occurred on apricot and which will be discussed later, gave additional evidence of the pathogenic abilities of certain pear cultures.

On December 19, 1935, *Phytomonas cerasi* var. *prunicola, P. cerasi,*

TABLE 4

RESULTS OF INOCULATING DUARTE PLUM TREES FEBRUARY 13, 1934

Organism	Inoculations producing symptoms, per cent	Length of cankers,* mm	Organism	Inoculations producing symptoms, per cent	Length of cankers,* mm
Phytomonas cerasi var. *prunicola* I	86	5–25	Bartlett I	86	5–44
Phytomonas cerasi var. *prunicola* II	86	10–29	Winter Nelis I	79	10–34
Phytomonas cerasi I	92	5–30	Winter Nelis II	83	5–25
Phytomonas cerasi II	100	20–30	Apple I	79	5–25
Wilder I	86	5–16	Apple II	39	5–10
Wilder II	79	5–20	*Phytomonas utiformica* "f"	66	5–25
Wilder III	79	10–25	Arkansas I	73	5–22
			Arkansas II	79	5–20
			Controls	0	0

* Measurements made 35 days after inoculation.

TABLE 5

RESULTS OF INOCULATING BLENHEIM APRICOT, PHILLIPS CLING PEACH, LAMBERT CHERRY, AND GRAND DUKE PLUM TREES ON JANUARY 21, 1935

Culture	Apricot		Peach		Cherry		Plum	
	Inoculations producing symptoms, per cent	Average length of cankers,* mm	Inoculations producing symptoms, per cent	Average length of cankers,* mm	Inoculations producing symptoms, per cent	Average length of cankers,* mm	Inoculations producing symptoms, per cent	Average length of cankers,* mm
Phytomonas cerasi var. *prunicola*	70	15	53	15	84	53	75	35
Phytomonas utiformica	91	24	83	23	60	32	70	14
Phytomonas citriputeale	74	17	60	30	60	26	60	43
Phytomonas papulans	0	0	0	0	0	0	0	0
Wilder I	70	14	67	17	48	24	30	15
Wilder II	64	20	38	25	47	23	45	13
Winter Nelis I	80	18	100	27	80	30	90	40
Winter Nelis II	66	19	50	15	50	16	20	13
Apple I	56	18	43	19	72	29	30	22
Arkansas I	87	25	74	21	72	32	65	25
Arkansas II	90	31	53	24	75	35	50	18
Controls	0	0	0	0	0	0	0	0

* Twenty inoculations into each of three trees. The measurements represent the average length of diseased areas along cambium and in bark 40 days after inoculation.

and *P. prunicola* Wor. were each inoculated at 100 places in five Duarte plum trees. Since the temperature did not favor rapid disease development during December and January the cankers produced averaged only 13 millimeters by January 19. Observations on March 4, however, showed that *P. prunicola* had produced cankers as extensive as had the other two organisms, some being 65 millimeters long and 30–35 millimeters wide.

On March 4, 1936, Wilder I and Winter Nelis I were inoculated into young Wilder pear trees. On the same date *Phytomonas cerasi* var. *prunicola,* culture Wilder I and culture Winter Nelis I, were inoculated into Bing sweet cherry. On March 9 examinations showed that in all cases the organisms were invading the bark tissues of both pear and cherry above and below the inoculation points. By March 14 the invaded zones in the pear trees were from 15 to 20 millimeters long and 10 to 15 millimeters wide. No difference existed in symptoms produced by cultures Wilder I and Winter Nelis I. On cherry the three cultures (*P. cerasi* var. *prunicola,* Wilder I, and Winter Nelis I) had produced diseased areas somewhat larger than on pear. Culture Winter Nelis I and *P. cerasi* var. *prunicola* produced somewhat larger cankers than did culture Wilder I, the average lengths being 31, 27, and 21 millimeters respectively.

Table 6 condenses the inoculation data to permit comparisons between results of different years. Whenever a particular culture was listed as pathogenic to a particular host, the character of the symptoms produced was considered in addition to the data on measurements. If on apricot, for example, the symptoms produced by a pear culture were not comparable with those caused by *Phytomonas cerasi* and *P. cerasi* var. *prunicola,* the results were listed as doubtful. This point is stressed because some cultures from pear produced on stone-fruit trees typical bacterial canker symptoms,[17] although the diseased areas were not always so large as those produced by *P. cerasi.*

The California cultures from pear limbs and culture Winter Nelis I from pear blossoms were pathogenic to the five species of stone fruits and to pear. The California cultures from pear blossoms were pathogenic to the stone fruits with but one exception, that of culture Winter Nelis II on European plum and on peach. A culture from apple (Apple I) was pathogenic to Japanese plum and apricot; but inoculations into European plum, peach, and sweet cherry were doubtful. A second culture (Apple II) was doubtful on Japanese plum, the only host into which it was inoculated. The Arkansas cultures were pathogenic to the five stone fruits. *Phytomonas utiformica,* in addition to pear, was pathogenic to

Japanese plum, peach, apricot, and sweet cherry; but doubtful results were obtained on European plum. *Phytomonas citriputeale* produced positive results on European plum, apricot, peach, and sweet cherry. No trials were made on pear. *Phytomonas cerasi* and *P. cerasi* var. *prunicola* were pathogenic to the stone fruits and also produced cankers in pear. *Phytomonas papulans,* on the other hand, produced no symptoms on any of the stone fruits.

TABLE 6

SUMMARY OF PATHOGENICITY STUDIES*

Culture	Source of culture		Japanese plum†	European plum	Apricot	Peach	Sweet cherry	Pear
	State	Host						
Phytomonas cerasi var. *prunicola* I	Calif.	Plum	+	+	+	+	+	+
Phytomonas cerasi var. *prunicola* II	Calif.	Apricot	+	+‡	+‡	+‡	+‡	..
Phytomonas cerasi I	Calif.	Apricot	+	+‡	+‡	+‡	+‡	+
Phytomonas cerasi II	Calif.	Peach	+	+‡	+‡	+‡	+‡	..
Phytomonas utiformica "f"	N. Y.	Pear	+	±	+	+	+	+
Phytomonas utiformica "r"	N. Y.	Pear	..	..	+	..	..	+
Phytomonas citriputeale	Calif.	Orange	..	+	+	+	+	..
Phytomonas papulans	East. U.S.	Apple	..	−	−	−	−	..
Wilder I	Calif.	Pear	+	+	+	+	+	+
Wilder II	Calif.	Pear	+	+	+	+	+	+
Wilder III	Calif.	Pear	+	..	..	..	..	+
Bartlett I	Calif.	Pear	+	..	..	..	..	..
Winter Nelis I	Calif.	Pear	+	+	+	+	+	+
Winter Nelis II	Calif.	Pear	+	±	+	±	+	..
Apple I	Calif.	Apple	+	+	+	±	±	..
Apple II	Calif.	Apple	±	..	..	..	..	..
Arkansas I	Ark.	Pear	+	+	+	+	+	..
Arkansas II	Ark.	Pear	+	+	+	+	+	..

* Key to symbols: +=pathogenic; −=nonpathogenic; ±=doubtful.

† This variety of plum (Duarte) is said to be *Prunus* (*salicina* × *munsoniana*) × *salicina*.

‡ These cultures had proved pathogenic to European plum, apricot, peach, and cherry in earlier tests.

Evidence of Natural Spread of Disease from One Host to Another.—A host that will develop symptoms when inoculated with bacteria pathogenic to another host will not necessarily contract the disease under field conditions. Evidence, however, points toward the spread of bacteria from stone-fruit trees to pear trees. In one pear orchard, for example, blossom blast occurred only adjacent to three badly diseased apricot trees. The blast was most abundant near the apricots, but was absent a few rows away. In another case, two-year-old pear trees adjacent to old diseased peach trees developed limb cankers, while those farther away remained healthy. One of the worst cases of pear blast observed occurred in a pear orchard interplanted with plums. The plums had apparently suffered from bacterial canker for some years. Although few limb

cankers were present in the pears, for several years blossom blast was prevalent, a fact indicating that the holdover source might have been the plums.

In connection with the inoculation results presented in table 5, attention was called to the development of secondary infections in apricot limbs inoculated with the following pear cultures: Wilder I and II, Winter Nelis I, and Arkansas II. The secondary infection probably did not result from bacteria coming from other trees, since the lesions occurred only below the inoculation points and since control punctures made at one side and above these limbs remained healthy. Healthy trees, furthermore, occurred to windward of those inoculated.

The following observation regarding possible spread of bacteria from pear to citrus should be recorded. In 1932, within two weeks after numerous new cankers appeared in an orchard of Wilder pears, typical citrus blast appeared in a row of orange trees bordering the orchard. No blast had occurred in these orange trees within the preceding three or four years; and close examination after the outbreak in 1932 failed to show any recognizable blast symptoms of previous years.

CULTURAL TESTS

Unless otherwise stated, the following tests were made at 25° C. All organisms made good growth at this temperature.

The synthetic medium used in most of the carbohydrate tests was the same as that designated basal medium 2 in an earlier article.[17] Its constitution was as follows: potassium dihydrogen phosphate 1.0 gram, magnesium sulfate 0.5 gram, potassium chloride 0.5 gram, sodium nitrate 2.0 grams, and ferrous sulfate 0.01 gram per liter. The pH was adjusted to 6.8 to 7.0 with sodium hydroxide.

Degree of Fluorescigenesis as a Distinguishing Feature.—The relations between the pathogenic bacteria that produce a green fluorescence were studied by Burkholder,[2] who attempted to adduce from his own and others' studies the degree of cultural homogeneity exhibited by species included in Bergey's genus *Phytomonas*. In many respects the fluorescent species constituted a closely related group, having common characteristics other than fluorescence. Burkholder's work encouraged Clara[4] to bring together and to study under the same conditions the cultural and pathogenic attributes of many of these species. Clara's conclusions will be reviewed later; he, as well as others, considers fluorescigenesis a cardinal diagnostic character.

The present author's study[17] of the stone-fruit canker indicated that the bacteria involved fell into two types or, as was finally concluded,

two strains or varieties. The type more commonly found on plum, cherry, and apricot differed from the less common type in not producing pigment on potato-dextrose agar. Other slight cultural differences were evident, the consistency of which will be considered later. The less common type was regarded as *Phytomonas cerasi* Griffin, while the more common was designated *P. cerasi* var. *prunicola* n. var.

While information was being secured on the presence of the pear bacterial canker in various localities, the large number of cultures obtained were seen to vary in their ability to produce pigment on potato-dextrose agar. Representative cultures, consequently, were compared with those from stone fruits in the following manner: The cultures were first grown for 24 hours in beef-extract broth and were then transferred to tubes of potato-dextrose agar. After a period extending to 27 days in some cases transfers were again made to beef-extract broth, and after 24 hours to potato-dextrose agar. In all, six such transfers to potato-dextrose agar were made. As the results showed, the organisms from pear separated themselves in the same manner as did the stone-fruit cultures. They consistently did or did not produce a green pigment on this medium. Of the 12 cultures from pear, 4 produced pigment and 8 did not. Of the 13 cultures from stone fruits, 5 produced pigment and 8 did not. The two cultures from apple (Apple I and Apple II) did not produce pigment, nor did those of *Phytomonas utiformica, P. citriputeale, P. prunicola* (Wormald) Bergey *et al.*, and *P. papulans*. Among those that produced pigment there was some variation; culture Wilder I, for example, produced a clear yellowish-green fluorescence in the medium, similar in every respect to that of *P. cerasi* I, whereas culture Wilder II produced at first a clear yellowish-green fluorescence, but after a few days a brownish discoloration of the agar. The same type of variation existed between *P. cerasi* I and *P. cerasi* II.

In order that later reference may be made to the ability of the individual cultures used in the inoculation experiments to produce pigment in potato-dextrose agar, the following list is given:

Fluorescent	*Nonfluorescent*	
Wilder I	Bartlett I	*Phytomonas citriputeale*
Wilder II	Winter Nelis I	*Phytomonas utiformica*
Wilder III	Winter Nelis II	*Phytomonas prunicola* Wor.
	Apple I	*Phytomonas papulans*
	Apple II	Arkansas I
		Arkansas II

The stone-fruit cultures designated *Phytomonas cerasi* are, of course, fluorescent, and those designated *P. cerasi* var. *prunicola* are nonfluorescent on potato-dextrose agar.

The difference in pigment production that distinguished *Phytomonas cerasi* and *P. cerasi* var. *prunicola* on potato-dextrose agar was not so clear-cut on certain other media,[17] being more a difference of intensity than of quality. Thus, in a synthetic liquid medium containing mannitol, glycerol, or sodium succinate as the energy source, a greenish-yellow pigment was produced by both, although that produced by *P. cerasi* was more intense. When cultures Wilder I, *P. cerasi*, *P. cerasi* var. *prunicola*, and *P. utiformica* were grown comparatively in the presence of various carbon sources (23 in all), culture Wilder I resembled *P. cerasi*, while *P. utiformica* resembled *P. cerasi* var. *prunicola* in fluorescigenesis.

Carbohydrate Utilization.—The medium used in these tests was basal medium 2, described earlier in this section. It soon proved ill-adapted to studies of increase in hydrogen-ion concentration when the carbon source used was not readily utilized by the bacteria. Trehalose and raffinose, for example, are less readily utilized than dextrose; yet growth is moderately luxuriant after about one week. If basal medium 2 is used, the pH is unchanged or is slightly increased. If, on the other hand, the basal medium is that proposed by the Society of American Bacteriologists *Manual*,[19] in which the nitrogen source is monobasic ammonium phosphate, the bacteria produce a definite decrease in pH when utilizing trehalose and raffinose. As will be mentioned later, a substitution of ammonium sulfate for sodium nitrate in basal medium 2 permits the hydrogen-ion concentration to increase more rapidly in the presence of dextrose as an energy source. Since the pH change is determined not only by the hydrogen or hydroxyl ions derived from the carbon source, but by the ions derived from all other constituents of the medium and by the buffering effects of the constituents, the conditions of the test must be specified. The basal medium used for the carbon-utilization tests reported in table 7 was basal medium 2. Despite the objectionable features of this medium in the presence of a poorly utilized carbon source, it supported a luxuriant growth and favored development of the fluorescent pigment. For those reasons it was used extensively.

As shown in table 7, the pear-canker organism (Wilder I) was grown comparatively with *Phytomonas cerasi* var. *prunicola*, *P. cerasi*, *P. prunicola* Wor., and *P. utiformica* on basal medium 2 in the presence of twenty-three carbon sources. *Phytomonas utiformica* was grown on all but three of these carbon compounds. The plus and minus signs represent greater acidity and greater alkalinity than the control tube after the bacteria had been growing for 10 days at 25° C. With but one exception the organisms produced the same type of reactions on all carbon

sources. The exception was in the case of rhamnose, where culture Wilder I had made visible growth although the others had not. This difference is not significant, since in further tests the other cultures produced visible growth after 14 days or so.

In later tests all the cultures used in the pathogenicity studies were grown comparatively on basal medium 2 with xylose, dextrose, and

TABLE 7

CARBOHYDRATE UTILIZATION*

Culture	Carbon source																												
	Xylose	Arabinose	Mannose	Dextrose	Levulose	Galactose	Sucrose	Maltose	Lactose	Trehalose	Raffinose	Rhamnose	Mannitol	Glycerol	Peptone	Sodium asparaginate	Sodium succinate	Sodium citrate	Sodium malate	Sodium lactate	Sodium tartrate	Sodium acetate	Sodium oxalate	Sodium formate	Starch
Phytomonas cerasi var. *prunicola* I	+	+	+	+	+	+	+	−	−	−	−	0	+	+	−	−	−	−	−	−	0	0	0	0	0
Phytomonas cerasi var. *prunicola* II	+			+			+																	0	0
Phytomonas cerasi I	+	+	+	+	+	+	+	−	−	−	−	0	+	+	−	−	−	−	−	−	0	0	0	0	0
Phytomonas cerasi II	+			+			+																	0	0
Phytomonas prunicola Wor	+	+	+	+	+	+	+	−	−	−	−	0	+	+	−	−	−	−	−	−	0	0	0	0	0
Wilder I	+	+	+	+	+	+	+	−	*t*	−	−	+	+	+	−	−	−	−	−	−	0	0	0	0	0
Wilder II	+			+			+																	0	
Wilder III	+			+			+																	0	
Bartlett I	+			+			+																	0	
Winter Nelis I	+			+			+																	0	
Winter Nelis II	+			+			+																	0	
Apple I	+			+			+																	0	
Apple II	*t*			*t*			*t*																	0	
Phytomonas utiformica I	+	+	+	+		+	+	−	−	−	−	0	+	+	−		−	−	−		0		0	0	0
Arkansas I	+			+			+																	0	
Arkansas II	+			+			+																	0	
Phytomonas citriputeale	+			+			+																	0	
Phytomonas papulans	+			+			+																	0	
Erwinia amylovora	0	+	+	+	+	+	+	+	0	+	+	0	0	0	−	−	0	0	0	0	0	0	0	0	0

* Key to symbols: + = change towards acid side; − = change towards alkaline side; *t* = growth but no change in pH; 0 = no growth.

sucrose as energy sources (10 grams per liter). With but one exception, that of culture Apple II in the presence of sucrose, all the organisms produced acid from the sugars (table 7). Although culture Apple II made a good growth on sucrose, no change in pH was visible after 10 days. In the degree to which the pH was changed, an apparent consistent difference was noticed on sucrose. Thus *Phytomonas cerasi* and the pear cultures that produced pigment on potato-dextrose agar caused less increase in hydrogen-ion concentration that did *P. cerasi* var. *prunicola* and those cultures (except Apple II) that did not produce pigment on potato-dextrose agar. In three experiments, for example, after 5 days the

latter group of cultures had reduced the pH to 3.8–4.1, whereas the former had reduced it only to 6.4–6.6. Under the conditions of these experiments at least, the cultures separated along the same lines as on potato-dextrose agar.

All the organisms were inoculated into tubes of basal medium 2 with formic acid (0.15 per cent, pH 6.8) as the only carbon source. None of

TABLE 8

Miscellaneous Cultural Features*

Culture	Gelatin liquefaction	Hydrogen sulfide production	Milk		Growth on nitrogen compounds			
			pH change	Peptonization	Ammonium sulfate	Sodium asparaginate	Asparagine	Glycine
Phytomonas cerasi var. *prunicola* I	+	–	Ak	+	+	+	+	+
Phytomonas cerasi var. *prunicola* II	+	–	Ak	–	+	+	+	+
Phytomonas cerasi I	+	–	Ak	+	+	+	+	+
Phytomonas cerasi II	+	–	Ak	+	+	+	+	+
Phytomonas prunicola Wor	+	–	Ak	+	+	+	+	+
Wilder I	+	–	Ak	+	+	+	+	+
Wilder II	+	–	Ak	+	+	+	+	+
Wilder III	+	–	Ak	+	+	+	+	+
Bartlett I	+	–	Ak	–	+	+	+	+
Winter Nelis I	+	–	Ak	+	+	+	+	+
Winter Nelis II	+	–	Ak	+	+	+	+	+
Apple I	+	–	Ak	+	+	+	+	+
Apple II	–	–	Ak	–	+	+	+	–
Phytomonas utiformica I	+	–	Ak	+	+	+	+	+
Arkansas I	+	–	Ak	+	+	+	+	+
Arkansas II	+	–	Ak	+	+	+	+	+
Phytomonas citriputeale	+	–	Ak	+	+	+	+	+
Phytomonas papulans	–	–	Ak	+	+	+	+	+
Erwinia amylovora	+	–	...	..	+	+	+	..

* Key to symbols: + = positive reaction or growth, as pertains to the respective headings; – = negative reaction or no growth; Ak = a shift of pH from 6.2 towards alkaline side.

the organisms made visible growth in this medium even after three weeks at 25° C. Apparently, therefore, formic acid is not an energy source that these bacteria can readily utilize (table 7).

Nitrogen Source.—The influence of the nitrogen source on the characteristics of the pH change produced by the bacteria has been mentioned. Sodium nitrate, though supporting an abundant growth, was less conducive to increase in hydrogen-ion concentration than monobasic ammonium phosphate. A further study of nitrogen sources revealed that ammonium sulfate, asparagine, sodium asparaginate, and glycine were utilized by all the bacteria (table 8). The characteristics of the pH change were as follows: on ammonium sulfate a rapid hydrogen-

ion increase occurred within a few days; on sodium nitrate and glycine the increase was much slower; on asparagine and sodium asparaginate the initial pH change was towards the alkaline side and was followed by a reversal only after several days. In the degree of pH change, *Phytomonas papulans* and culture Apple II differed from the rest on sodium nitrate and glycine.

Nutrient-Dextrose Broth.—This medium was beef-extract broth to which had been added 10 grams of dextrose per liter. The pH was adjusted to 6.8. By the end of 72 hours all the bacteria except *Phytomonas papulans* and culture Apple II had produced a dense uniform turbidity of the medium and a slight, easily fragmented surface film. *Phytomonas papulans* and culture Apple II differed from the rest in producing a tough, membranous surface film and very little turbidity.

Colony Characteristics on Potato-Dextrose Agar.—The consistency, topography, and internal structure of potato-dextrose agar colonies varied greatly even in *Phytomonas cerasi* I, the descendant of a single-cell isolation.[17] On the whole, the colonies of all the cultures except *P. papulans* and culture Apple II were similar, being after 48 hours from 1.5 to 3 millimeters in diameter. The margins were either entire or slightly lobed. The topography was flat or slightly raised. The consistency was butyrous, except as reported earlier for *P. cerasi;*[19] the color slightly bluish to white. The internal structure was amorphous or broken by dark, wavy lines extending in a general radial direction. Wormald[21] has reported this last-named feature to be characteristic of his *P. prunicola.* The colonies of *Phytomonas papulans* and culture Apple II differed from the rest in being more opaque and somewhat slimy.

Liquefaction of Gelatin.—Stab cultures were incubated at 21° C, observations being made at 2-day intervals. The cultures of *Phytomonas cerasi* were the first to begin liquefaction, followed by those of *P. cerasi* var. *prunicola* (table 8). By the end of 168 hours all cultures, except Apple II and *P. papulans,* had produced a stratiform liquefaction to a depth of 1 inch or more. The tubes were then placed at 25° C, but culture Apple II and *P. papulans* failed to start liquefaction after 3 days at this temperature.

Reaction in Milk.—Enough litmus was added to one lot of skimmed milk to produce a distinct blue. To another lot was added enough brom thymol blue to give a distinct color. The tubes were sterilized by steaming at atmospheric pressure for 20 minutes on 4 successive days. The final pH was approximately 6.2.

In five experiments the initial reaction of all cultures was an increase

in alkalinity (table 8). Clearing of the milk accompanied by a distinct odor of peptonization began in most cases within 4 or 5 days. In this reaction *P. cerasi* was slightly more rapid than the rest. *Phytomonas papulans*, culture Apple II, and culture Bartlett I were distinguishable from the rest by their failure to produce a peptonization after 11 days.

Hydrogen Sulfide Production.—Beef-extract agar was prepared as recommended by the Society of American Bacteriologists *Manual.*[10] None of the bacteria under study produced hydrogen sulfide in this medium (table 8).

Starch Hydrolysis.—Two types of media were employed for these tests: (1) beef-extract agar plus 10 grams of starch per liter and (2) basal medium 2 plus 10 grams of starch per liter (table 8). Beside *Phytomonas cerasi, P. cerasi* var. *prunicola,* and *P. prunicola* the only other culture used was Wilder I. Good growth but no starch hydrolysis was made on the former medium; no growth was made on the latter.

Malachite Green Agar.—The growth of the bacteria on malachite green agar is reported because further differentiation of *Phytomonas papulans* and Apple II was obtained. To basal medium 2 were added 10 grams of dextrose, 15 grams of agar, and malachite green (1:100,000); the pH was adjusted to 6.8. In petri dishes this medium was distinctly green.

Phytomonas papulans and culture Apple II were differentiated from the rest because *P. papulans* failed to grow and culture Apple II grew only slightly. Although culture Bartlett I and *P. cerasi* var. *prunicola* II grew somewhat more slowly than the rest, they conformed to the characteristics of a majority of the others—namely: (1) a flat, butyrous, opalescent growth, (2) later a greenish-yellow pigment that stained the bacterial mass and diffused into the medium (*P. cerasi* I and II produced pigment earlier and in greater intensity), and (3) gradual disappearance of the malachite green after about 2 or 3 days so that by the end of 10 days the plates were usually cleared of the stain.

Differentiation of Erwinia Amylovora and the Green-Fluorescent Organisms.—Since some rapid method of distinguishing *Erwinia amylovora* from the pear-canker organism was desirable, the bacillus was included in the studies of fluorescigenesis and carbon-source utilization. Although the blast and canker cultures produced pigment on basal medium 2 in the presence of a number of carbon compounds, *E. amylovora* showed no indication of producing pigment. According to table 7, under the conditions of these tests, *E. amylovora* was distinguishable from the green-fluorescent organisms when growing on a number of carbon sources. The most rapid method of differentiating these organ-

isms, however, would consist in adding to basal medium 2, glycerol, mannitol, or peptone, carbon compounds especially favorable to pigment production.

Besides cultural methods, figure 6 shows that *Erwinia amylovora* may be distinguished from the fluorescent organisms by inoculations

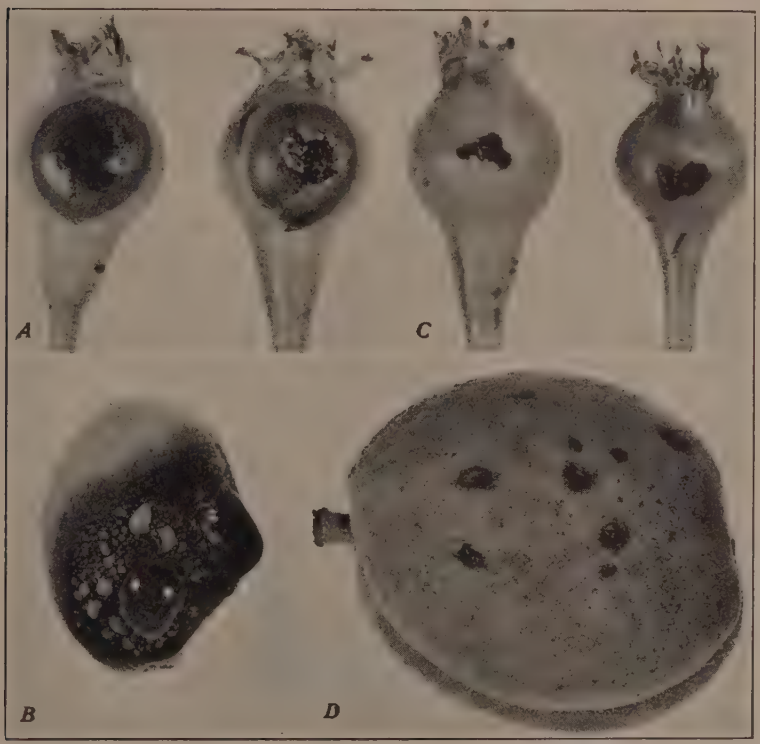

Fig. 6.—Difference in symptoms produced on pear and apricot by *Erwinia amylovora* (*A, B*) and by the pear-canker and blast organism (*C, D*). *Phytomonas cerasi* var. *prunicola* produced the same symptoms as did the pear-canker bacterium.

into young pear and apricot fruits. The fire-blight organism readily involved the entire fruit, producing a white, slimy exudate, while the pear-blast and stone-fruit organisms produced around the needle punctures only small, black, sunken pits and no visible exudate. Smith and Fawcett[13] have obtained with *Phytomonas citriputeale, P. syringae,* and *P. cerasi* the same type of black pits on a variety of fruits.

DISCUSSION

The literature contains considerable evidence that certain organisms included in this study are closely related. Although much of this evidence has been summarized elsewhere,[10] some recapitulation is necessary.

As Bryan,[1] Smith,[11] and Smith and Fawcett[13] have shown, *Phytomonas citriputeale* C. O. Smith is similar to if not identical with *P. syringae* (Van Hall) Bergey *et al.* As the last-named authors further showed, *P. cerasi*[5] is also closely related to these organisms. Elliott[6] has since considered *P. citriputeale* but not *P. cerasi* synonymous with *P. syringae.*

Both Smith[12] and the present writer[10] have questioned the status of *Phytomonas prunicola,* after Wormald's description of it as a distinct species. Smith considered it to be similar to if not identical with *P. citriputeale,* whereas the writer found it identical with an organism he considered to be a strain or variety of *P. cerasi*—namely, *P. cerasi* var. *prunicola.*

In 1932 Clara[3] described *Phytomonas utiformica* as the cause of blossom blight, fruit spot, and leaf spot of pears in New York. The same year Rosen[9] found a similar blossom disease on pears in Arkansas; and a year later Rosen and Bleeker[10] published comparative serological and pathological tests in which they included their organism from pear, a culture of *P. syringae,* and a culture of *P. prunicola.* They concluded that these organisms were identical and questioned the advisability of considering *P. cerasi, P. papulans, P. nectarophila* (Doidge) Bergey *et al., P. barkeri* (Berridge) Bergey *et al.,* and *P. utiformica* as species separated from the lilac organism, *P. syringae,* by significant differences.

Clara[4] compared a number of green-fluorescent organisms, including *Phytomonas cerasi, P. utiformica,* and *P. syringae.* On the basis of pathogenicity to seventeen hosts he concluded that these organisms were related, but believed that certain cultural differences warranted separating them into species.

Dunegan[5] has recently reported results of inoculating peach with *Phytomonas syringae* Van Hall, *P. prunicola* Wor., *P. mors-prunorum* (Wormald) Bergey *et al.*[21] *P. papulans,* a bacterium from apple target canker, and a bacterium from leaf spot of Italian prune. He found that when any of these bacteria were injected into leaves and green shoots, chlorotic or bleached areas surrounded by purplish zones were devel-

[5] Smith and Fawcett's culture of *P. cerasi* was obtained in California. After corresponding with Smith the writer is convinced that it was *P. cerasi* var. *prunicola.* For description of this variety see the writer's earlier publication.[17]

oped, symptoms differing from those produced by *P. pruni* (E. F. Smith) Bergey *et al.*, on this host.

We see, therefore, in the works of most of these authors a tendency towards clearing the literature of certain species created as the result of studying each on a limited number of hosts. Such a tendency is justifiable as far as it is founded upon a direct comparison of the organism in question. The interlinking evidence supplied by the works of Bryan,[1] Smith and Fawcett,[13] Smith,[12] Rosen and Bleeker,[10] and the writer[10, 17, 18] furnishes reasons for considering *P. syringae, P. citriputeale, P. cerasi* var. *prunicola,* and *P. prunicola* as either identical or differing only to a slight degree. Clara[4] alone maintains that *P. syringae* and *P. cerasi*[6] are distinct species, although he considers *P. citriputeale* and a number of other green-fluorescent pathogens synonymous with *P. syringae.*

If we examine Clara's[4] reasons for regarding the three species as distinct, we see that they pertain both to pathogenic and to cultural features. He found, for example, that they differed in their ability to produce lesions on such diverse plants as *Trifolium repens, Holcus* sp., *Zea mays,* and *Syringa vulgaris.* On thirteen other hosts, including *Pyrus communis* and *Prunus avium,* the three organisms were indistinguishable. His results with *Syringa vulgaris* differ from those of Smith and Fawcett[13] inasmuch as he found *P. cerasi* nonpathogenic to this host, whereas Smith and Fawcett obtained infection.

In cultural tests Clara found the three organisms very similar in many respects, but different in their reaction on certain carbon compounds—namely, raffinose, glycerol, salicin, acetic acid, and formic acid. Thus he reported that *Phytomonas utiformica* was the only one to "ferment" raffinose, salicin, and formic acid; that *P. cerasi* and *P. utiformica* but not *P. syringae* fermented acetic acid, whereas *P. utiformica* and *P. syringae* but not *P. cerasi* fermented glycerol. His conclusions regarding the failure of *P. cerasi* to ferment glycerol are contrary to those of Smith and Fawcett[13] and of the writer,[17] who found this compound to be an excellent energy source. Likewise his findings regarding failure of *P. cerasi* to ferment raffinose and salicin do not conform with the writer's earlier results[17] nor with the studies presented herein, which indicate that these compounds supported fair growth. In the present study *P. utiformica* failed to make visible growth on a medium containing formic acid in the same concentration as used by Clara; acetic acid was not used.

As this brief review shows, the majority of the workers consider the organisms used in this study very closely related, and Clara's dissenting

[6] The culture supplied Clara was that of *Phytomonas cerasi* var. *prunicola.*

views are based upon much evidence directly contrary to that of the others.

We may now consider the results of the present work. Though certain gaps exist in the inoculation data and though cultural tests are by no means complete, the studies have yielded certain evidence:

1. The California cultures from pear differed consistently among themselves in one respect—production of fluorescent pigment on potato-dextrose agar. That is, the three cultures from limb canker of Wilder pear produced fluorescence on potato-dextrose agar, whereas those from twigs and blossoms did not, a characteristic similar to that separating *Phytomonas cerasi* from *P. cerasi* var. *prunicola*. The few instances in which the pear organisms varied in other respects could easily have resulted from experimental error.

2. The mutual pathogenic abilities, the parallel fluorescigenic variability on potato-dextrose agar, and the similar reactions in all other tests exhibited by the stone-fruit and California pear cultures give no indication that they are very different.

3. Apple II culture, although belonging to the green-fluorescent group, is definitely different from the pear cultures and from Apple I; the latter appears to be very similar to the pear cultures.

4. The culture furnished the writer by Roberts and provisionally designated by him as *Phytomonas papulans* is distinctly unlike any of the other organisms tested herein. Dunegan,[5] so far as known, is the only worker who has compared *P. papulans* with one of those included in the present study.

5. *Phytomonas citriputeale, P. utiformica,* and the pear-blast cultures from Arkansas were pathogenically similar to the California pear cultures, and to *P. cerasi* and *P. cerasi* var. *prunicola* when inoculated into five species of stone fruits. *Phytomonas utiformica* was, furthermore, shown to produce the same type of symptoms on pear as did the California pear-canker organism. As far as the cultural studies went, this first-named group of organisms agreed with *P. cerasi* var. *prunicola* and with those California pear cultures that were not fluorescent on potato-dextrose agar.

This summary indicates that the only clear cases of differences within this group of cultures were those of *Phytomonas papulans* and Apple II. This is true as far as both the pathogenic and cultural tests are concerned. The rest of the cultures, however, though identical in inoculation tests, exhibited on media certain differences that should be mentioned. We saw that the three California cultures from pear-limb canker (Wilder I, II, and III), when grown on potato-dextrose agar, in their

production of pigment resembled *P. cerasi*, whereas the remaining California pear cultures as well as *P. citriputeale*, *P. utiformica*, and cultures Apple I and II, in their failure to produce pigment on potato-dextrose agar, resembled *P. cerasi* var. *prunicola*. As earlier comparisons[17] had shown, *P. cerasi* and *P. cerasi* var. *prunicola* differed slightly in other tests. When these tests were undertaken for the present study, the similar slight differences were again evident: *P. cerasi*, for example, began to peptonize milk and to liquefy gelatin somewhat earlier than did *P. cerasi* var. *prunicola*. The pear cultures that resembled *P. cerasi* on potato-dextrose agar, on the other hand, were not so distinguished from those that resembled *P. cerasi* var. *prunicola*. Another example of separation of the cultures was afforded by sucrose in basal medium 2. Here *P. cerasi* and the three pear cultures that resembled it on potato-dextrose agar (Wilder I, II, and III) produced a smaller decrease in pH than did *P. cerasi* var. *prunicola* or any of the other cultures except *P. papulans* and culture Apple II.

Hence, except in the cases of *Phytomonas papulans* and culture Apple II, the only consistent separations of cultures were in their fluorescent capacities on potato-dextrose agar and in the degree to which they changed pH in the presence of sucrose. Host source, on the other hand, did not appear to be important as a line of cleavage. Particularly can this be said of the two stone-fruit organisms, of *P. utiformica*, of culture Winter Nelis I, and of culture Wilder I, all of which were inoculated into pear as well as five species of stone fruits. How many more slight differences can be obtained by increasing the number of tests and by refining the technique can only be surmised. Unquestionably, a number of small differences would be regarded by some as justifying the continuation of the names of existing species and the use of new names for those unnamed organisms included herein. The final disposition will, of course, depend upon more complete studies and upon the prevailing conception of species limits.

Provisionally, at least, the evidence justifies including in one species *Phytomonas cerasi*, *P. cerasi* var. *prunicola*, *P. citriputeale*, *P. utiformica*, Rosen's organism, and the California pear organism. These organisms are unquestionably very closely related to *P. syringae*. The fact that there is no recorded variation of *P. syringae* comparable with that separating *P. cerasi* and *P. cerasi* var. *prunicola* cannot be overlooked. If any changes are made, *P. cerasi* should probably retain a varietal rank.

SUMMARY AND CONCLUSIONS

The major object of this work was to establish the relation between a canker and blossom blast of pear and the bacterial canker of stone-fruit trees. Both the pear canker and pear blossom blast were known to be caused by bacteria that were on standard culture media similar to each other and to *Phytomonas cerasi,* cause of the stone-fruit bacterial canker. By observing the diseases of the two hosts for a number of seasons, information was obtained concerning the season of activity, the parts of the hosts attacked, and the character of the symptoms. To determine pathogenic similarities, the bacteria were inoculated at various times into *Pyrus* sp. and into five species of *Prunus.* By cultural tests the bacteria were compared as to growth on various standard media, growth in special media containing different carbohydrate and nitrogen sources, reactions in milk, liquefaction of gelatin, and production of hydrogen sulfide. Besides the two strains of stone-fruit organisms (*P. cerasi* and *P. cerasi* var. *prunicola*) and bacteria from pear limb cankers and blossom blast, the pathogenicity and cultural studies included the following organisms: *P. utiformica, P. papulans, P. citriputeale,* cultures obtained by Rosen from pear blossoms in Arkansas, and cultures obtained by the writer from apple in California. Incidentally, *Erwinia amylovora* was carried through certain of the cultural tests, and a method for differentiating it from the canker organisms is described.

The results of these studies afforded the following conclusions: First, the limb canker and blossom blast of pear are phases of the same disease, which also attacks dormant buds, twigs, and fruit. Second, the pear and the stone-fruit diseases exhibit similarities as to parts of the host attacked, character of symptoms, and season of activity. The bacteria from the two hosts were, furthermore, identical in the inoculation and cultural tests. Third, the inoculation and cultural tests support the view that *Phytomonas utiformica, P. citriputeale,* and the bacterium furnished by Rosen are identical with the stone-fruit organism. The bacterium recently isolated by Roberts and designated *P. papulans* is an unrelated species.

In the writer's opinion, therefore, these organisms, except of course *Phytomonas papulans,* should be given the same species name. The preponderance of evidence in the literature points towards *P. syringae* as the correct binomial.

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INHERITANCE OF RESISTANCE TO POWDERY MILDEW IN BEANS

BJARNE DUNDAS

INHERITANCE OF RESISTANCE TO POWDERY MILDEW IN BEANS[1, 2]

BJARNE DUNDAS[3]

POWDERY MILDEW (*Erysiphe polygoni* D.C.) frequently causes considerable loss in beans (*Phaseolus vulgaris* L.) grown in the coastal districts of California. Among the numerous bean varieties grown in test plots at Berkeley each season by W. W. Mackie, of the Division of Agronomy, differences in varietal susceptibility have been apparent.

In the summer of 1932 the mildew was very abundant at Berkeley, but the Pinto and some other varieties were not infected. The following winter crosses were made in the greenhouse between these and certain other varieties with the idea of studying the inheritance of resistance and introducing resistance into commercial varieties which lack it.

METHODS EMPLOYED IN INOCULATION TESTS

The reaction of the bean plants to mildew was determined by inoculating detached leaflets floated on a sugar solution in petri dishes, a method used by Yarwood[(4)][4] in his work with the powdery mildew of red clover. This method has numerous advantages. Plants may be grown in the greenhouse and tested at any time of the year, and uniformity of inoculum and environmental conditions during inoculation tests are insured. The same single-spore culture of the mildew was propagated in petri dishes free from contamination by other strains of mildew and was used in all of the inoculation tests, which extended over a period of more than three years.

Length of Life of the Detached Leaflets.—Tests made to determine what substrate was most favorable to prolonged life of the detached bean leaflets showed that a 10 per cent solution of sucrose was superior to several higher and lower concentrations tested and to pure water or Hoagland culture solution. In petri dishes leaves floated on a 10 per cent sucrose solution or lying on cotton saturated with this solution remained alive (turgid and normal green in color) two to three weeks at room temperature and over a month at 8° C with a maximum of 55 days at 8°, 12 days at 25°, and 7 days at 31°. Time of day of collection of the

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[4] Superscript numbers in parentheses refer to Literature Cited at end of this paper.

leaflets seemed to be unimportant. Leaves lived about two days longer in light than in the dark. The third youngest unfolded leaf was found to be similar to older leaves in its reaction to mildew and was generally used. In their mildew reaction, leaves from greenhouse plants were similar to those from field plants. Infection with the mildew was found to shorten the life of the detached leaflets by 2 to 8 days.

Optimum Conditions for Production of Inoculum and for Infection.—Spores of powdery mildew produced in the light germinated somewhat better than those produced in the dark, and young spores from colonies 3 to 6 days old germinated much better than those from colonies over 19 days old. Spores produced under relative humidities[2, 3] of 8 per cent (over KOH), 33 per cent (over $MgCl_2 \cdot 6H_2O$) and 79 per cent (over NH_4Cl) germinated about equally well and somewhat better than those produced at 0 (over P_2O_5) or 100 per cent relative humidity.

It was found that spores would germinate on dry slides as well as on water. Germination of dry spores (25–77 per cent) on dry slides occurred promptly at temperatures between 8° and 25° C, at ordinary room humidities as well as in saturated air and in darkness or in light. In fact, a low percentage of spores germinated at temperatures as high as 28° and 31° C and at room temperature in a relative humidity of 8 per cent. No spores germinated at approximately zero relative humidity.

The time of day when spores were applied to the leaflets made no difference in the resultant growth of mildew. On leaflets on 10 per cent sugar solution the mildew developed equally well in the dark and in the light, but on weaker or stronger sugar solutions it developed better in the light. It developed well at 15° C and at room temperature (about 21°) but less abundantly at 25° and not at all at 31°. At 8°, 22 days were required for it to reach a stage of development such as that arrived at in 8 or 9 days at room temperature. It was found to develop well over a wide range of relative humidity.

Inoculation of Leaves.—Inoculum was produced on leaflets on 10 per cent sugar solution in petri dishes stored in the light at room temperatures, and the spores were used when the colonies were 6 to 10 days old.

In order to determine the relative susceptibility of individual plants, the third youngest unfolded leaf was placed in a petri dish on cotton soaked with a 10 per cent sucrose solution with the upper epidermis uppermost. To obtain spores for inoculation, heavily mildewed portions of a leaflet were cut out and held with a forceps over the leaflet to be inoculated, and by tapping the forceps the spores were dislodged. Or the spores were deposited by gently touching the leaflet with the mil-

dewed leaf fragment. The dishes containing the inoculated leaflets were then stored in the light at room temperatures, and readings of mildew severity were made after 7 to 9 days, and again on resistant leaflets two weeks after inoculation. Very little difficulty was caused by contamination with molds. To supplement such tests, potted plants in the greenhouse were also occasionally inoculated.

Scale of Severity of Infection.—The severity of the mildew was estimated on a scale of 0 to 4 as follows:

0: No mycelium; spores germinate and may cause small necrotic spots by killing a few epidermal cells.

t (trace): Scant mycelium present; no spores; colonies few; necrotic spots present.

1: Mycelium scant; a few spores formed; colonies few; necrotic spots present.

2: Mycelium more abundant; spores formed rather generally on the mycelium; necrotic spots present.

3: Mycelium and spores fairly abundant; very few necrotic spots.

4: Mycelium and spores very abundant, covering the leaflet; spores formed within 4 days.

Readings between 0 and 2 designate resistance; 3 and 4, susceptibility.

RELATIVE SUSCEPTIBILITY OF BEAN VARIETIES TO MILDEW

The relative severity of mildew on certain varieties of beans in the field as a result of natural infection and in the greenhouse as a result of inoculation of potted plants is compared in table 1 with the severity of mildew on inoculated detached leaflets of the same varieties. Inoculation of the detached leaflets is shown to be a more severe test than is afforded by field or greenhouse infection. In no instance did field or greenhouse infection occur on varieties showing less than a 2 reaction in the dish test. Numerous inoculation tests were made and the results in table 1 are typical.

The varieties Hungarian, Lady Washington, Pinto, Yellow, and Pink, were resistant; Frijole negros, Long Roman, and *Phaseolus vulgaris* 5003, intermediate; and Robust and Red Kidney susceptible. The Pink variety was slightly less resistant than the other four in that group, and in the seedling stage was much more susceptible than in later stages. Three other resistant varieties also showed a trace of infection in the seedling stage.

Reaction of Parental Material—the Pinto, Long Roman, and Robust Varieties.—As parental material for a study of the inheritance of mil-

dew resistance, the varieties Pinto, Long Roman, and Robust were selected. Pinto (4369) has always given a negative reaction in dish tests. In 1932, 1933, and 1935 no mildew developed on it in the field, but in 1934 a small amount developed owing to the presence of a new physiologic form of the mildew that year. Long Roman (4521) has been susceptible in dish tests, usually giving a reading of 3. In the field in 1932 no infection appeared before late in the fall when most varieties

TABLE 1

MILDEW SUSCEPTIBILITY OF CERTAIN VARIETIES AS INDICATED BY THE DISH TEST, BY NATURAL INFECTION IN THE FIELD, AND BY INOCULATION OF PLANTS IN THE GREENHOUSE, 1932–1933

	Variety‡	Mildew readings*						
		Field 1932	Grown in greenhouse 1932–1933		Grown in field 1933			Field notes 1935†
			Plant† inoculation	Dish test, old	Field†	Dish test		
						Seedling	Old	
Resistant	Hungarian (4404)	0	0	0	0	0	0	0
	Lady Washington [84(213)32]	0	0	0	0	*t*	0	0
	Pinto (4369)	0	0	0	0	0–*t*	0	0
	Yellow (4429)	0	0	0	0	*t*	0	0
	Pink (4436)	0	0	0–1	0	3–4	0–1	0
Semi-resistant	Frijole negros (5033)	0	*m*	2	0	3	2–3	*m*
	Long Roman (4521)	*t*	*m*	3	*m*	3–4	3	*m*
	Phaseolus vulgaris (5003)	*t*	*m*	3	*m*	3	3	*m*
Susceptible	Robust (4458)	3	*m*	4	*m*	4	3–4	*m*
	Red Kidney (4764)	4	*m*	4	*m*	4	4	*m*

* Field notes for 1934 are not included because a different strain of mildew was present which attacked the resistant varieties.

† It is merely indicated whether mildew developed (*m*) or not (0).

‡ Numbers given in this column are accession numbers of the California Experiment Station.

were harvested. In 1933 and 1935 a very small amount of infection was present, but in 1934 it was more severely infected. Robust (4458) from 1932 to 1935 showed a light to medium-heavy field infection. It is highly susceptible in the dishes, usually with a reading of 4.

MILDEW REACTION OF THE F_1, F_2, AND F_3 PROGENIES OF ROBUST × PINTO

Crosses between resistant and susceptible varieties were made in the greenhouse in the winter of 1932–33. The F_1 generation was grown in the field the following summer. F_2 generations have been grown both in the greenhouse and in the field. Only in the field has enough seed for conclusive F_3 tests been obtained. F_3 generations have been grown in the

greenhouse. For routine work the F_2 and F_3 were mostly planted in sterile soil in 6-inch pots, 6 seeds in each. Less damping-off was experienced in sterile soil than in nonsterile soil. To insure prompt germination, the seed was scarified. The temperature in the greenhouse has been about 60° to 70° F.

The F_1 plants of the Robust × Pinto cross and their parents were grown in the field. The mildew reaction was determined by the dish tests both in the seedling and in older stages. The F_1 hybrids and their resistant Pinto parent were about equally resistant, as shown in table 2. Some additional F_1 plants grown in the greenhouse were tested approxi-

TABLE 2

MILDEW INFECTION ON ROBUST AND PINTO BEANS AND THEIR F_1 AND F_2 PROGENIES

Parent or cross	Number of plants with the mildew infection indicated						Total	$\frac{D}{E}$*
	0	*t*	1	2	3	4		
Robust	0	0	0	0	3	15	18	....
Pinto	18	0	0	0	0	0	18	....
F_1	5	1	0	0	0	0	6	....
F_2	121	9	0	0	9	34	173	0.07

* $\frac{D}{E} = \frac{\text{Deviation from expected ratio number}}{\text{Probable error of the number of the population}}$

mately every week during the first two months, and it was found that the mildew reaction of young plants was 0–1 and of older plants, almost always 0, like that of the resistant parent. The younger stages of Pinto showed somewhat more resistance than the younger stages of F_1 hybrids, a fact which indicates that the complete pair of factors for resistance is more potent than a single one.

For the F_2 generation, the progenies from two F_1 plants were observed separately, but since the two sets presented no genetic differences they are treated together. One primary leaf from each F_2 plant was tested for mildew susceptibility 7 to 10 days after the seedlings emerged and one leaf from each plant was tested when the plants were mature. The two tests were found to agree, so that in this cross the early test was reliable and susceptibility did not decrease with age. Furthermore the ratios of resistant to susceptible were the same in the plants tested twice as in those that had died before the second test was made. The results are included in table 2.

F_1 plants of Robust × Pinto may give a reading of *t* at the age of two months. This approximates the age of the F_2 plants at the second reading. For the resistant parent, Pinto, a reading of *t* was not obtained later

TABLE 3

Mildew Reading of F_3 Plants of a Robust × Pinto Cross

F_2 plant No.	Number of F_3 plants with readings						Ratio resistant / susceptible	$\frac{D}{E}$	Families in F_2 group
	0	t	1	2	3	4			
4	24	9	..	..	..	..	33:0	....	12 homozygous resistant
6	27	7	1	..	..	..	35:0	....	
10	21	9	3	..	..	..	33:0	....	
13	31	3	..	..	..	..	34:0	....	
16	28	7	..	..	..	..	35:0	....	
18	26	8	..	..	..	..	34:0	....	
21	21	5	..	..	..	..	36:0	....	
25	25	8	..	..	..	..	33:0	....	
30	27	7	..	..	..	..	34:0	....	
36	30	5	..	..	..	..	35:0	....	
38	24	2	..	..	..	..	26:0	....	
41	29	4	..	..	..	..	33:0	....	
1	..	..	..	..	17	15	0:32	....	10 homozygous susceptible
8	..	..	..	..	10	24	0:34	....	
14	..	..	..	..	4	18	0:22	....	
19	..	..	..	..	..	17	0:17	....	
27	..	..	..	..	5	26	0:31	....	
33	..	..	..	..	3	14	0:17	....	
35	..	..	..	..	17	18	0:35	....	
42	..	..	..	..	7	25	0:32	....	
43	..	..	..	..	8	22	0:30	....	
45	..	..	..	..	4	23	0:27	....	
2	17	7	1	..	3	3	25:6	1.07	23 heterozygous resistant
3	9	18	1	..	4	3	28:7	1.01	
5	11	13	3	..	3	4	27:7	0.88	
7	9	12	3	..	7	4	24:11	1.30	
9	16	9	1	..	4	4	26:8	0.29	
11	16	3	..	..	5	..	29:5	2.06	
12	5	10	5	..	5	..	22:5	1.15	
15	13	13	..	..	6	4	26:10	0.57	
17	2	14	11	1	2	6	28:8	0.57	
20	13	10	2	..	5	4	27:7	0.88	
22	20	7	..	..	3	4	27:7	0.88	
23	17	10	..	..	9	..	27:9	0.00	
24	23	2	..	..	4	2	25:6	1.07	
26	14	9	3	..	5	5	25:10	0.72	
28	17	8	..	..	3	5	25:8	0.15	
29	19	6	..	..	9	..	25:9	0.29	
31	13	10	2	..	1	6	25:7	0.61	
32	14	7	..	..	4	4	21:8	0.48	
34	15	5	..	..	6	1	20:7	0.16	
37	9	10	4	1	5	7	24:12	1.71	
39	20	8	..	..	5	3	28:8	0.57	
40	14	6	..	..	6	3	20:9	1.11	
44	18	7	2	..	3	3	27:6	1.34	

than 16 days after planting; later readings were all 0. The F_2 hybrids which in the second test showed a reading of *t* may thus be heterozygous rather than homozygous for resistance, while those that gave a reading of 0 may be either homozygous or heterozygous. Although a definite distinction between plants homozygous and heterozygous for resistance was not established, they are both in the resistant group, which by lack of plants in the intermediate classes 1 and 2 is distinctly separated from the susceptible group. The ratio 130:43 fits almost perfectly a 3:1 ratio and indicates that resistance to mildew in Pinto is due to a single dominant Mendelian factor.[a]

From each of 45 F_2 plants (the progeny of one F_1 plant) grown in the field in 1934, 36 seeds were planted in the greenhouse the following winter. The mildew reaction of the F_2 parents was unknown. The mildew reaction of each plant of the 45 F_3 progenies was determined by dish tests in the young stage. The results, including the ratio of resistant to susceptible plants in each family, are given in table 3. The F_3 families are grouped on the basis of the readings, and it may be seen that the 45 parental F_2 plants may be classified as follows: homozygous resistant, 12; heterozygous resistant, 23; homozygous susceptible, 10. This is a ratio of 35 resistant to 10 susceptible. The $\frac{D}{E}$ is 0.64. This approximates a 1:2:1 ratio or a ratio of 3 resistant : 1 susceptible, as was found in the tests with the F_2 generation, and establishes that the resistance in Pinto is due to a main single Mendelian factor pair.

There is a rather wide variation in the readings of the 23 heterozygous F_3 families. For instance family 17 has the average of its 28 resistant readings between *t* and 1 with two plants in the 0 class and one in the 2 class, while family 22 has 20 readings of 0 and 7 of *t*, with none in classes 1 and 2. This indicates that there are factors or combinations of factors present which modify the resistance. Although some of the heterozygous F_3 families have more plants with intermediate readings than did the F_2 generation as shown in table 2, there is nevertheless a distinct difference between the resistant and susceptible plants.

MILDEW REACTION OF THE F_1, F_2, AND F_3 PROGENIES OF LONG ROMAN × PINTO

It will be recalled that Pinto is resistant and Long Roman is somewhat resistant in the field but shows susceptibility in the dish tests. The seven F_1 plants were grown in the field, and when tested in the dishes all were resistant to the mildew and gave a reading of 0, like the resistant parent Pinto (table 4).

An F_2 generation was grown in the greenhouse. Tests made when the plants were young showed more variation in mildew resistance than did tests made when the plants were older, and a few plants changed from susceptible when young to resistant when older. The readings given in table 4 represent the average of 7 to 10 individual leaves taken from each plant at different times and tested by the dish method. The constancy of the later readings and the rather distinct separation of resistant and

TABLE 4

Mildew Infection on Long Roman and Pinto Beans and Their F_1 and F_2 Progenies

Parent or cross	Number of plants with the mildew infection indicated						Total	$\frac{D^*}{E}$
	0	*t*	1	2	3	4		
Grown in greenhouse								
Long Roman	0	0	0	0	9	0	9	....
Pinto	9	0	0	0	0	0	9	....
F_2	45	29	9	0	8	10	101	2.47
Grown in field								
Long Roman	0	0	0	0	13	0	13	....
Pinto	13	0	0	0	0	0	13	....
F_1	7	0	0	0	0	0	7	....
F_2	53	15	13	4	12	9	106	1.83

* $\frac{D}{E} = \frac{\text{Deviation from expected ratio number}}{\text{Probable error of the number of the population}}$

susceptible groups by the absence of class 2 as shown in table 4 indicate that the F_2 generation should give a reliable picture of the inheritance of resistance. Although there are a larger number of readings of *t* and 1 than in the Robust × Pinto F_2 hybrids, the results, like those of the previous cross, indicate the presence of a main factor pair for resistance in the Pinto variety.

F_2 plants from seed from the same F_1 plant used for those grown in the greenhouse were also grown in the field in 1934. Seedlings were tested in the dishes for mildew resistance within 4 days after emergence and a second test was made when the plants began to bloom. The readings on the older plants are given in table 4. They give about the same fit to a 3:1 ratio of susceptible to resistant as the greenhouse series. A few plants changed from susceptible in the seedling stage to resistant when older. There are 4 plants with a reading of 2, and class 3 is relatively larger than in the greenhouse series. A similar difference between field and greenhouse-grown plants has been observed in other trials.

The F_3 generation was grown from seed from 47 resistant and 10 susceptible F_2 plants grown in the field in 1934, including the 4 plants with

TABLE 5

MILDEW READINGS OF F_2 PLANTS AND THEIR F_3 PROGENY FROM A LONG ROMAN × PINTO CROSS

F_2		F_3							
Mildew reading	Plant No.	Number of plants with mildew readings						Ratio resistant : susceptible	D^* / E
		0	*t*	1	2	3	4		
0	1	28	6	0	1	0	0	35:0	....
	2	24	8	2	0	0	0	34:0	....
	3	25	3	0	0	0	0	28:0	....
	4	19	8	2	1	0	0	30:0	....
	5	34	1	0	0	0	0	35:0	....
	6	21	7	2	3	0	0	33:0	....
	7	13	4	3	7	0	0	27:0	....
	8	33	1	0	0	0	0	34:0	....
	9	27	2	0	0	0	0	29:0	....
	10	31	2	0	0	0	0	33:0	....
	11	14	1	0	0	4	0	15:4	0.59
	12	26	0	0	0	4	3	26:7	0.74
	13	22	6	1	0	4	1	29:5	2.06
	14	40	2	0	0	8	5	42:13	0.35
	15	19	1	2	0	6	0	22:6	0.65
	16	28	2	0	0	6	0	30:6	1.71
	17	26	1	0	0	6	2	27:8	0.43
	18	23	6	0	0	7	0	29:7	1.14
	19	24	1	0	1	7	0	26:7	0.74
	20	20	0	0	0	5	0	20:5	0.86
	21	17	2	0	0	5	4	19:9	1.29
	22	19	6	0	0	8	2	25:10	0.72
t	23	39	0	0	0	0	0	39:0	....
	24	39	0	0	0	0	0	39:0	....
	25	19	6	3	0	0	0	38:0	....
	26	25	10	0	1	0	0	36:0	....
	27	27	8	1	0	0	0	36:0	....
	28	24	3	0	2	1†	0	29:1	....
	29	18	5	1	0	7	2	24:9	0.45
	30	32	0	0	0	7	0	32:7	1.51
	31	30	1	0	0	5	4	31:9	0.54
	32	22	1	0	0	7	1	23:8	0.15
	33	19	8	0	0	8	1	27:9	0.00
	34	30	8	0	0	9	1	40:10	1.21
1	35	25	1	0	0	7	0	26:7	0.74
	36	22	3	0	0	5	1	25:6	1.07
	37	19	1	1	0	2	8	21:10	1.38
	38	18	2	1	1	4	10	22:14	2.86
	39	20	10	0	1	4	1	21:4	1.54
	40	20	3	0	0	4	1	23:5	1.29
	41	36	1	0	1	7	1	38:9	1.38
	42	28	4	2	1	14	0	35:14	0.86
	43	19	2	0	0	6	5	21:11	1.82
2	44	16	9	1	3	7	12	39:19	2.03
	45	16	7	0	0	4	4	23:8	0.15
	46	14	8	0	0	0	6	22:6	0.65
	47	14	5	2	0	5	4	21:11	1.82
3	48	0	0	0	0	5	10	0:15	..
	49	0	0	0	0	10	12	0:22	...
	50	0	0	0	0	4	18	0:22	....
	51	0	0	0	0	15	8	0:23	...
4	52	0	0	0	0	11	5	0:16	.. .
	53	0	0	0	0	10	15	0:25	....
	54	0	0	0	0	12	12	0:24	....
	55	0	0	0	0	12	23	0:35	....
	56	0	0	0	0	2	16	0:18	....
	57	0	0	0	0	7	15	0:22	....

* $\frac{D}{E}$ = $\frac{\text{Deviation from expected ratio number}}{\text{Probable error of the number of the population}}$

† Thought to be the result of a mixture.

a mildew reading of 2 (table 4). The F_2 plants were the progenies of one F_1 plant. The populations of the 47 F_3 families from resistant F_2 plants range from 19 to 58 with only 9 below 30. The susceptible F_2 plants (mildew reading of 3 or 4) yielded a comparatively small amount of seed owing to the injury from mildew, and the population of their F_3 families ranges from 15 to 36.

Of the 47 resistant F_2 plants (readings 0, *t*, 1, and 2) tested, 16 proved to be homozygous and 31 heterozygous for resistance, a result which is very close to the 1 : 2 ratio expected from a random sample. The 31 heterozygous F_2 plants segregated in the F_3 in accordance with the single-factor hypothesis. The susceptible F_2 plants (readings 3 and 4) gave only susceptible progeny. The resistance observed in Long Roman has not interfered with the expression of the Pinto resistance in this test.

The F_2 readings give an indication as to the homozygosity or heterozygosity of the plants in that F_2 mildew readings of 1 and 2 all represented heterozygous plants, while readings of 0 and *t* represented both homozygous and heterozygous plants in about equal numbers.

SUMMARY

For use in determining the susceptibility of beans (*Phaseolus vulgaris* L.) to powdery mildew (*Erysiphe polygoni* D.C.), the mildew was grown in petri dishes on detached bean leaflets supported on cotton soaked in a 10 per cent sucrose solution. Use of the dish-culture method permitted the continuous use of one physiological race of the mildew and offered numerous other advantages.

The susceptibility of different varieties and individual plants was determined by inoculating detached leaves. The results were in most cases found to be in close agreement with field and greenhouse infection. In no case was infection less severe in the dishes. Mildew readings were made on a scale of 0 to 4.

The varieties Hungarian, Lady Washington, Pinto, and Pink were found to be resistant, and Robust and Red Kidney were susceptible, as tested in the dishes and in field and greenhouse. Frijole negros was resistant in the field and greenhouse, but semiresistant in the dishes. Long Roman was semiresistant in the field and greenhouse and susceptible in the dishes.

The F_1, F_2, and F_3 progenies of a cross between the susceptible Robust and the resistant Pinto were tested by the dish method. Of the 45 F_2 plants tested, 12 proved to be homozygous resistant, 23 heterozygous resistant, and 10 homozygous susceptible.

The F_1, F_2, and F_3 progenies of a cross between the semiresistant

Long Roman and the resistant Pinto were similarly tested and of the 47 resistant F_2 plants tested, 16 proved to be homozygous and 31 heterozygous. Readings of 0 and *t* in the F_2 plants indicated homozygosity or heterozygosity for resistance; 1 and 2, heterozygosity only; and 3 and 4, homozygosity for susceptibility.

In the crosses with Robust and Long Roman, the Pinto is seen to have a single Mendelian factor pair for resistance to the strain of powdery mildew used.

Plants were often somewhat more susceptible in the seedling stage than later.

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SPOTTED WILT OF THE SWEET PEA

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SPOTTED WILT OF THE SWEET PEA[1]

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INTRODUCTION

IN THE COASTAL COUNTIES of California the sweet pea, *Lathyrus odoratus* L., is frequently afflicted with a streak disease. The cause of the disease is sometimes obscured by viruses of the mosaic type or by resemblance to the description of bacterial streak of sweet pea.[5] [4] It has, however, been proved to be a virus identical with that responsible for the spotted wilt disease of tomato. Recently[6] this virus has been reported as the cause of a streak disease of the garden pea, *Pisum sativum* L.

SYMPTOMS

A characteristic symptom of the disease on sweet pea is a necrotic streaking of stems and petioles. The streaks are reddish brown to dull purple in color and in the advanced stage are conspicuous. They may run the entire length of the stem, parallel to the long axis, being found on analysis to consist of disconnected short streaks or a continuous long one. They have been seen to develop both above and below the point of virus inception.

Leaves and shoots may turn yellow and die. Early symptoms on the foliage appear as spots, usually 5 to 15 mm long, more or less circular or oval in shape, yellowish at first, with diffuse margins. Later the spots become somewhat brownish and form a pattern typical of the virus on other hosts. From these spots are developed yellowish sectors or zones, ordinarily one or only a few on each leaf. The spotting of the foliage is fairly definite in early stages, although not striking; but the general yellowing which may follow becomes a conspicuous symptom. Symptoms of the disease are shown in figure 1, *A* and *D*.

Blossoms on infected plants have been seen to develop a circular pattern in the pigment and the virus has been recovered from such material. Again, blighting of the whole shoot may occur prior to blossom formation.

Variability in the expression of symptoms has been observed. Plants infected early may yellow and die without showing other symptoms. In other cases the virus may be limited to local lesions instead of becoming

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[4] Superscript numbers in parentheses refer to Literature Cited at end of this paper.

Fig. 1.—Symptoms of the spotted-wilt virus: *A*, Yellowish foliage spots on sweet pea. *B*, Brown foliage spots and blighting on garden pea. *C*, Pitted necrosis of pod of garden pea. Ovules failed to develop in this pod. *D*, Dark reddish-brown streak on stem of sweet pea. *E*, Circular pattern on pod of garden pea (×2).

systemic. This is frequently the case in artificial inoculations wherein lesions remain confined to the zone immediately above and below the inoculated leaves. Such infection does not result in the severe stunting and destructiveness obtained with systemic infections unless, as sometimes happens, the stem is so weakened that it breaks at the point of inoculation.

INOCULATION EXPERIMENTS

The diseased sweet-pea plants first used successfully as inoculum in the artificial transmission of the virus and in its determination were obtained in November. They were taken from a severe infestation of streak in a greenhouse devoted to sweet-pea culture at San Pablo, where spotted wilt is prevalent. The plants, only a few inches aboveground when infected, showed stem streaking, spotting of the foliage, general yellowing, and blighting of the shoots. They were free from mosaics. In some cases symptoms were confined to the blighted shoot, since subsequent shoots developed on the same root seemed to be normal for the time being. In other instances the virus became systemic, and where such plants survived to produce bloom, they sometimes bore flowers showing pigment patterns in the petals, as described above.

Expressed juice from the naturally infected sweet peas was inoculated by means of the carborundum rubbing method[3] into healthy sweet peas and test plants for the spotted wilt virus. In four trials with fresh collections of the diseased sweet peas, the virus was successfully transmitted into *Nicotiana glutinosa* L., *N. tabacum* L., *Datura stramonium* L., and *Lycopersicum esculentum* Mill. Local lesions obtained on *N. glutinosa* and *N. tabacum,* as well as systemic infections induced in these and in *D. stramonium* and *L. esculentum* were all typical for the spotted wilt virus.

The sweet-pea plants which became infected resembled the original material, and the virus was recovered from them in turn on the test plants. Although transmission of the virus from sweet pea to the other hosts used in these trials was obtained fairly readily, the percentage of sweet-pea plants which became infected was low, varying from 0 to 60 per cent. This is in line with experience in other instances where the spotted wilt virus has been concerned. The incubation period varied usually from 14 to 30 days.

These results indicate that the spotted wilt virus of tomato was present in the diseased sweet peas, and that it could be recovered on other host plants by the mechanical method of transmission used.

By reversing the procedure of inoculation, healthy sweet peas (Red Boy variety) were inoculated from a tomato plant containing the spotted

wilt virus. Streak symptoms appeared on 4 plants out of 13 inoculated. Inoculations with the virus from other host plants of the spotted wilt virus gave similar results in repeated tests, although in still other cases negative results were obtained. A trial with naturally infected Romaine lettuce, *Lactuca sativa* L. var. *longifolia* Lam., grown near the greenhouse where the disease was found on sweet pea, produced 8 infections out of a total of 30 inoculated sweet-pea plants. This appeared to identify further the spotted wilt virus in the field with that in the greenhouse. The lower transoms of the greenhouse had been left open after planting to sweet peas, ample opportunity thus being offered for thrips, the known vector of the spotted wilt virus, to enter in large numbers. Also, at that season the disease was spreading rapidly in nearby fields of head lettuce, *L. sativa* var. *capitata* L.

The results of these experiments show that by mechanical inoculations and cross-inoculations it is possible to produce symptoms typical of spotted wilt of tomato and streak of sweet pea, irrespective of the virus source. Sweet peas naturally infected with streak were used on the one hand, and plants inoculated by known cultures of the spotted wilt virus on the other. Check plants used in the experiments remained healthy.

To parallel the work with sweet pea, attempts were being made from time to time to transmit a streak disease of garden pea (*Pisum sativum*) occasionally found in the field. In December, 1935, a number of plants showing streak symptoms were found in San Luis Obispo County in a winter planting of peas grown on the coast. The plants showed brown necrotic spots on the foliage, darkening of the veins, reddish-brown to purple streaks on the stems, and blighting of the tops. Pods showed circular spotting or, in advanced stages, a pitted necrosis. Death of the pods was marked by a purplish-bronze cast. These symptoms, since described by Whipple,[6] are illustrated in figure 1, *B, C,* and *E*.

With the juice from these plants, spotted wilt symptoms were obtained on *Nicotiana glutinosa* and *N. tabacum,* and in addition 2 out of 16 inoculated sweet-pea plants developed streak. Inoculation of the spotted wilt virus into Perfection peas from infected tomato yielded 4 infections out of a possible 13; from infected tobacco 5 out of 10 plants were infected; and from Romaine lettuce 10 out of 31 showed systemic infections, while 20 of the 31 showed local lesions. These and other similar data obtained from cross-inoculations with the pea streak and spotted wilt virus have confirmed Whipple's results and conclusions upon the identity of these viruses.

Transmission of the spotted wilt virus to sweet pea was also obtained by means of thrips. Infective larvae and adults of *Thrips tabaci* Lind.,

obtained from head lettuce infected with the spotted wilt virus, were caged on healthy sweet peas in the greenhouse. In the first series, 4 out of 34 plants became infected, typical symptoms of the disease appearing in 20 days. In the second series 3 out of 31 plants were infected, symptoms appearing in 30 days. At the same time the virus was successfully transmitted by thrips to garden pea in confirmation of Whipple's work.

DISCUSSION

Symptoms on sweet pea resembling a streak have been reported by Zaumeyer and Wade[7] after inoculation with mosaic viruses from white sweet clover and white clover. The virus involved in both cases may be Pierce's[2] white clover virus 1.

The streak disease of sweet pea reported here is now recognized as an important factor in sweet-pea culture in certain coastal districts of California, and probably occurs in other regions where the spotted wilt virus is present. Its seriousness may be felt especially where sweet peas are planted in the vicinity of spotted wilt host crops in these districts at seasons when large migrations of the thrips vector may take place. Because of the long list of common ornamentals known[1] to harbor the virus, garden plots of sweet peas are particularly liable to infection, owing to the many likely sources of the virus at hand at all times of the year. In the writers' experience it is frequently impractical to grow sweet peas to a satisfactory conclusion in private gardens in infested districts. When infected early, the plants fail to grow as they should, turn yellow, and, if they do not die, are lacking in productiveness and quality.

Circumstances attending the disease may hinder immediate diagnosis. There may be only a partial display of the symptoms, and where streaking only is evident, it resembles a bacterial disease. Tissue plantings on agar media from necrotic stem lesions were sterile except for occasional colonies of bacteria in two lots of material cultured. These bacteria, when sprayed on healthy plants in a humidity chamber, produced no infection.

A common complication of the streak disease is caused by mixtures with mosaic viruses. Among other virus diseases Stubbs'[4] enation mosaic of pea (pea virus 1) and Pierce's[2] common pea mosaic (pea virus 3) are found on sweet pea in California. Where a mosaic and streak occur together the tendency has been to attribute the severity of the symptoms to a high virulence of the mosaic virus, or to a mixture of mosaic viruses. However, it has been possible to isolate both the spotted wilt virus and a mosaic virus from plants showing the combined symptoms, by means of differential hosts. Also, the pea aphid (*Macrosiphum pisi* Kalt), not be-

ing a vector of the spotted wilt virus, may be used to isolate the mosaic virus where mosaic and spotted wilt are present in the same plant. In mechanical inoculations with expressed juice the mosaics are usually transmitted in a much higher percentage of cases than spotted wilt. As a result only the mosaic virus may be recovered when the juice from a plant containing both viruses is applied mechanically to a common host. Other streaks of pea, however, may be more readily transmitted by mechanical inoculation.

Control of streak would seem to lie in the isolation of sweet pea plantings from crops susceptible to spotted wilt and from infested districts, or in the protection of plants from migrations of infective thrips.

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